

## Research Paper

# Varietal differences in yield and nutritional quality of alfalfa (*Medicago sativa*) accessions during 20 months after planting in Ethiopia

## *Diferencias varietales en producción y calidad nutritiva de accesiones de alfalfa (Medicago sativa) en Etiopía*

TESSEMA TESFAYE ATUMO<sup>1</sup> AND CHRISTOPHER STEPHEN JONES<sup>2</sup>

<sup>1</sup>Arba Minch Agricultural Research Center (AMARC), Arba Minch, Ethiopia. [www.sari.gov.et](http://www.sari.gov.et)

<sup>2</sup>International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia. [www.ilri.org](http://www.ilri.org)

### Abstract

Feed supply in terms of quality and quantity plays an important role in livestock production and productivity. Here we report on varietal differences in yield and nutritional quality among 9 alfalfa accessions over 7 harvests following planting in Ethiopia. Experimental design was a randomized complete block with 3 replications at Chano Mille, Southern Ethiopia on a sandy loam soil where mean annual rainfall is 544 mm. Days to harvesting, plant height, dry matter yield, seed yield and the concentrations of the nutritional quality parameters crude protein (CP), neutral detergent fiber, acid detergent fiber, acid detergent lignin, hemicellulose and cellulose plus in vitro dry matter digestibility (IVDMD) and relative feed value (RFV) were assessed to rank the accessions. There were significant ( $P < 0.001$ ) differences between accessions and harvests in plant height, dry matter yield and seed yield. Accession ILRI\_7323A performed best in all agro-morphological aspects. All accessions, except 1, produced forage with CP in excess of 30% and IVDMD greater than 80% with RFV greater than 150 at 50% flowering, indicating the high quality of forage produced. Further studies to assess the longevity of stands of the various accessions seem warranted along with studies in higher rainfall environments or under irrigation.

**Keywords:** Crude protein, digestibility, fiber, forage legume, quality, yield.

### Resumen

La disponibilidad de forraje en términos de calidad y cantidad tiene un papel importante en la producción y productividad del ganado. En un experimento conducido en un suelo franco arenoso en Chano Mille, al sur de Etiopía, se determinaron las diferencias varietales en rendimiento y calidad nutritiva entre nueve accesiones de alfalfa en siete cosechas después de la siembra. La precipitación media anual en la zona es de 544 mm. Los tratamientos fueron dispuestos en un diseño de bloques completos al azar con tres repeticiones. Se evaluaron los siguientes parámetros: días hasta la cosecha, altura de planta, rendimiento de materia seca (MS) y las concentraciones de proteína cruda (PC), fibra detergente neutra, fibra detergente ácida, lignina detergente ácida, hemicelulosa y celulosa, y la digestibilidad in vitro de la materia seca (DIVMS). Para clasificar las accesiones, se utilizó el valor relativo del forraje (VRF). La altura de planta y el rendimiento de MS y de semilla presentaron diferencias significativas ( $P < 0.001$ ) entre accesiones y cosechas. La accesión ILRI\_7323A mostró el mejor comportamiento en todos los aspectos agromorfológicos. Cuando las plantas presentaban 50% de floración, todos los materiales, con excepción de una accesión, produjeron forraje con una concentración de PC superior al 30%, DIVMS superior al 80% y un VRF superior a 150, lo que indica la alta calidad del forraje. Se discute la necesidad de estudios complementarios para evaluar la longevidad de las accesiones y su comportamiento en ambientes de mayor precipitación o bajo riego.

**Palabras clave:** Digestibilidad, fibra, leguminosa forrajera, proteína cruda, valor nutritivo.

Correspondence: T.T. Atumo, Arba Minch Agricultural Research Center (AMARC), PO Box 2228, Arba Minch, Ethiopia.  
Email: [tessema4@gmail.com](mailto:tessema4@gmail.com)

## Introduction

Livestock production is highly dependent on the production and availability of quality feed and forage resources (Thornton 2010). According to the Central Statistical Agency (CSA 2018), total livestock population in Ethiopia is estimated to be 193.23 million units (excluding beehives), with cattle making up 60.39 million, sheep 31.3 million, goats 32.74 million, horses 2.01 million, donkeys 8.85 million, mules 0.46 million, camels 1.42 million and poultry 56.06 million. The livestock sector produces 15–17% of Ethiopia's GDP, 35–40% of agricultural GDP and 37–87% of household incomes (Gebremariam et al. 2013). The major impediment to improved production of the Ethiopian livestock sector is considered to be insufficient feed, in terms of both quality and quantity (Tesfay et al. 2016). To help combat this situation and reduce the nutritional constraints to livestock production, the use of adapted, high-yielding, drought-tolerant improved forages of high quality is recommended (Derseh et al. 2016; Bashe et al. 2018).

Alfalfa (*Medicago sativa*) is a high-yielding, perennial (2–3 years with highest economical yield) forage legume that is well suited to hay, silage or pasture production and is known as the “Queen of Forages” for its excellent quality, especially in terms of high crude protein concentration. This species can tolerate frequent harvesting (as frequent as every 35–40 days) by storing energy in the crown to support re-growth after cutting (Undersander et al. 2011). It adds nitrogen to the soil via symbiotic nitrogen fixation in bacterial nodules on the roots, and can also withstand long periods of water deficit by halting vegetative growth and accessing water from deep in the soil through its deep root system (Annicchiarico and Pecetti 2010). These properties make

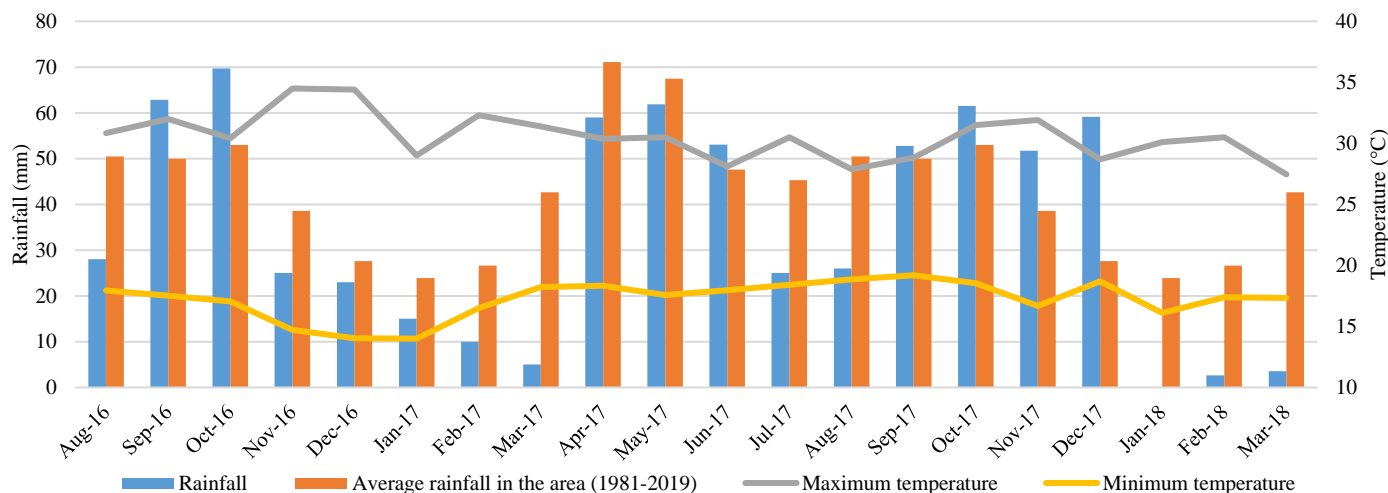
alfalfa one of the most widely grown forage crops in the world.

The project reported here focused on the adaptability, forage yield, seed yield and nutritional quality of 9 alfalfa accessions, selected in consultation with the forage genebank manager of the International Livestock Research Institute (ILRI), based on previous experience and consideration of which accessions may be most suitable to perform well in the selected environment, and planted in Arba Minch Agricultural Research Center, Southern Ethiopia.

## Materials and Methods

Varietal evaluation of yield and nutritional quality of 9 accessions of alfalfa (*Medicago sativa*) was conducted between September 2016 and February 2018 at the Arba Minch Agricultural Research Center, Chano Mille substation (6°06' N, 37°35' E; 1,206 masl), where mean annual rainfall is 544 mm. Weather data including mean monthly rainfall and maximum and minimum temperatures during the course of the trial are presented in Figure 1.

Laboratory analysis of a composite (0–30 cm) soil sample collected from the experimental site (Chano Mille) revealed that soil texture is a sandy loam, with pH 6.2, available phosphorus 14.5 g/kg, total nitrogen 0.29%, organic carbon 1.19%, organic matter 1.63% and potassium 1.12 cmolc/kg. The pH of the experimental soil is within the range for productive soils (FAO 2000) and the soil is considered medium in available P, medium to high in N fertility class (Tadesse et al. 1991) and in the medium range for organic carbon (Herrera 1995), which makes it satisfactory for producing good growth and yields of alfalfa.



**Figure 1.** Rainfall and temperature data for Arba Minch area, Gamo Gofa Zone, Ethiopia during the study.

The experiment involved 9 alfalfa accessions (Table 1) with 3 replications and was laid out in a randomized complete block design. Seed was sown in plots (3 × 2 m), consisting of 9 rows of 3 m with 20 cm between rows, using a seed drill on 6 October 2016 during the main cropping season. All recommended field management practices and packages, e.g. weeding, fertilizer application [100 kg/ha NPS (19% N:38% P<sub>2</sub>O<sub>5</sub>:7% S) fertilizer as basal], were performed uniformly over all plots for the duration of the trial. K application was not recommended by the Ethiopia Soil Information Service (EthioSIS) for this location, so none was applied.

**Table 1.** Alfalfa accessions used in the study.

Accession	Cultivar name	DOI
ILRI_14176A	WL 514	<a href="https://doi.org/10.18730/FSNAR">10.18730/FSNAR</a>
ILRI_15585A	WL 516	<a href="https://doi.org/10.18730/FTTW5">10.18730/FTTW5</a>
ILRI_5680A	Moopa	<a href="https://doi.org/10.18730/G5AZY">10.18730/G5AZY</a>
ILRI_5681A	wild	<a href="https://doi.org/10.18730/G5B0Z">10.18730/G5B0Z</a>
ILRI_7323A	wild	<a href="https://doi.org/10.18730/G6C2G">10.18730/G6C2G</a>
ILRI_7369A	Kohli	<a href="https://doi.org/10.18730/G6D7G">10.18730/G6D7G</a>
ILRI_9234A	Siriver	<a href="https://doi.org/10.18730/G7R6P">10.18730/G7R6P</a>
ILRI_9237A	Sheffield	<a href="https://doi.org/10.18730/G7R9S">10.18730/G7R9S</a>
ILRI_9239A	Trifecta	<a href="https://doi.org/10.18730/G7RBV">10.18730/G7RBV</a>

Seven consecutive harvests were conducted with the criterion for harvesting being when 50% of plants were flowering. The central 5 rows were harvested at 7 cm above ground level with a hand sickle and data collected included: plant height; dry matter yield; and nutritional quality parameters. Briefly, for plant height measurement a total of 10 plants from each plot were randomly selected and measured from ground to the top of the plant just prior to forage harvesting. Following harvesting, green forage was gathered and weighed with a spring balance to determine the fresh matter yield (FMY) per plot. A 500 gram sample from each plot was then placed in a pre-weighed cloth bag and oven dried at 105 °C for 12 h to a constant weight. Dry matter yield (DMY) was calculated using the formula  $DMY (t/ha) = DM\% \times FMY/ha$ . Dried samples were then preserved for subsequent analysis of nutritional quality parameters. Of the remaining 4 rows the outer 2 rows were discarded as border rows, while the other 2 rows were used to obtain seed yields. The border rows were harvested with the seed rows after the forage rows were harvested to minimize the impact of any border effect on the seed rows. Plants from these rows were cut to 20 cm above ground level, taking care to avoid loss of pods and seed, and plants were threshed to obtain seed. The seed was dried in the shade to a constant weight to calculate seed yield.

Forage nutritional quality, in terms of ash, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) concentrations, was

assessed in the laboratory according to National Forage Testing Association procedures (Undersander et al. 1993). Relative feed value (RFV) was calculated using the formula:  $RFV = DDM (\% \text{ of DM}) \times DMI (\% \text{ of BW}) / 1.29$  (Saha et al. 2010), where DDM is the digestible dry matter derived from ADF [ $DDM = 88.9 - (0.779 \times ADF)$ ] and DMI is dry matter intake as a % of body weight (BW) derived from NDF ( $DMI = 120/NDF$ ). Hemicellulose and cellulose concentrations of the accessions were calculated by the formulae: neutral detergent fiber (NDF) – acid detergent fiber (ADF); and ADF – acid detergent lignin (ADL), respectively.

The data generated were statistically analyzed using the analysis of variance procedure and least significance difference, at the 5% probability level, of Genstat statistical software (Version 16, VSN International Ltd, UK) and correlation analysis was undertaken using the SAS (2004) software package.

## Results

The first harvest of alfalfa accessions was conducted 104 days after planting (18 January 2017), with subsequent harvests a further 140, 48, 44, 33, 44 and 92 days (on 7 June, 25 July, 7 September, 10 October and 23 November 2017 and 23 February 2018, respectively) after the previous harvest. This wide range in harvest cycles reflected the plants' ability to re-grow given the available soil moisture and temperature in this dryland system (Figure 1), which represents what happens in the fields of smallholder farmers in the region. The overall means for plant height and DM yield of the 9 alfalfa accessions for the 7 harvests are shown in Tables 2 and 3, respectively.

Plant height varied significantly ( $P < 0.001$ ) between accessions, being greatest for Accession ILRI\_7323A (70.0 cm) followed by ILRI\_9239A (66.8 cm), while the shortest accession was ILRI\_14176A (57.4 cm) (Table 2). Mean DM yields also varied significantly ( $P < 0.05$ ) among the accessions, with the highest and most consistent yield being recorded for accession ILRI\_7323A (4.99 t DM/ha) followed by ILRI\_5680A (4.73 t DM/ha), while the lowest yield was recorded for ILRI\_14176A (3.86 t DM/ha) (Table 3). Mean DM yields at different harvests varied significantly ( $P < 0.05$ ), which was not surprising given the different lengths of inter-harvest intervals plus variation in rainfall and temperature for the different periods. Highest DM yields were recorded in September, October and June and the lowest in July (Table 3). Seed yields (SY) of alfalfa accessions over the course of the experiment (Table 4) varied significantly ( $P < 0.001$ ) among accessions. Pooled mean values showed that the highest SY was recorded for genotype ILRI\_7323A (107.7 kg/ha), while the lowest was for ILRI\_9237A (32.9 kg/ha).

**Table 2.** Mean herbage plant height (cm) of 9 alfalfa accessions across 7 harvests following planting in October 2016 in Ethiopia.

Accession	Jan 17	Jun 17	Jul 17	Sep 17	Oct 17	Nov 17	Feb 18	Mean
ILRI_14176A	54.8	68.5	36.0	56.3	68.3	58.3	59.6	57.4d
ILRI_15585A	57.5	76.0	39.1	68.0	69.3	63.3	67.9	63.0bc
ILRI_5680A	59.3	76.7	43.5	59.5	76.5	63.1	71.5	64.3bc
ILRI_5681A	52.7	69.2	45.2	65.6	79.7	61.2	57.2	61.5cd
ILRI_7323A	63.3	81.7	55.3	73.7	83.5	67.1	65.5	70.0a
ILRI_7369A	55.4	65.8	40.8	65.0	60.3	52.7	65.8	58.0d
ILRI_9234A	57.5	69.7	37.5	60.8	75.7	59.4	70.3	61.6cd
ILRI_9237A	58.1	72.5	32.7	61.2	71.7	64.6	69.8	61.5cd
ILRI_9239A	63.7	78.7	43.9	61.7	82.5	59.3	77.9	66.8ab
Mean	58.1d	73.2a	41.6e	63.5c	74.2a	61.0cd	67.3b	62.7

CV (%) = 11.1. LSD<sub>0.05</sub>: accessions = 4.24, harvests = 3.74. Means within columns and rows with a common letter are not significantly different (P>0.05).

**Table 3.** Mean dry matter yields (t/ha) of 9 alfalfa accessions across 7 harvests following planting in October 2016 in Ethiopia.

Accession	Jan 17	Jun 17	Jul 17	Sep 17	Oct 17	Nov 17	Feb 18	Total yield	Mean
ILRI_14176A	2.68	4.52	2.23	4.61	4.51	3.16	5.29	27.0	3.86d
ILRI_15585A	3.46	4.99	2.43	5.31	4.47	2.91	5.19	28.8	4.11cd
ILRI_5680A	3.66	5.74	3.29	5.58	6.02	3.76	5.05	33.1	4.73ab
ILRI_5681A	3.08	5.36	3.06	5.63	5.58	3.47	5.48	31.7	4.52abc
ILRI_7323A	3.51	4.84	3.97	6.26	6.80	4.25	5.29	34.9	4.99a
ILRI_7369A	3.39	4.97	3.17	4.56	4.71	3.76	5.29	29.9	4.27bcd
ILRI_9234A	3.42	4.67	2.57	4.76	4.64	3.52	5.78	29.4	4.19cd
ILRI_9237A	3.34	6.35	2.70	4.85	4.71	3.52	5.68	31.2	4.45bc
ILRI_9239A	4.06	4.92	3.01	4.95	5.02	3.64	5.15	30.8	4.39bc
Mean	3.40b	5.15a	2.94c	5.17a	5.16a	3.55b	5.35a	30.7	4.39

CV (%) = 17.6. LSD<sub>0.05</sub>: accessions = 0.47, harvests = 0.42. Means within a column and row with a common letter are not significantly different (P>0.05).

**Table 4.** Mean seed yield (SY, kg/ha) and crude protein yield (CPY, t/ha) of 9 alfalfa accessions across 7 harvests following planting in October 2016 in Ethiopia.

Accession	SY (kg/ha)	CPY (t/ha)
ILRI_14176A	44.2de	1.68bcd
ILRI_15585A	86.1b	1.77bcd
ILRI_5680A	40.8ef	1.25e
ILRI_5681A	45.3de	1.53d
ILRI_7323A	107.7a	1.84abc
ILRI_7369A	58.9c	1.63cd
ILRI_9234A	52.1cd	2.06a
ILRI_9237A	32.9f	1.69bcd
ILRI_9239A	43.6de	1.91ab
Mean	56.9	1.70
LSD <sub>0.05</sub>	9.94	0.27
CV (%)	10.2	9.0

Mean values within columns with a common letter are not significantly different (P>0.05).

Mean values for forage quality traits for the 9 alfalfa accessions across the 7 harvests in terms of ash, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) concentrations plus in vitro dry matter digestibility (IVDMD) and relative feed value (RFV) are presented in Table 5. There was significant (P<0.05) variation among accessions in terms of CP concentration, IVDMD and RFV. All accessions except ILRI\_5680A recorded CP concentration overall in excess of 30% and all accessions recorded IVDMD in excess of 80%. Crude protein yields over the total period ranged from 1.25 (ILRI 5680A) to 2.06 (ILRI\_9234A) t/ha (Table 4). RFV varied from 160 to 184. No significant variation (P>0.05) was detected in concentrations of ash, neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), hemicellulose and cellulose.

**Table 5.** Mean chemical composition, in vitro dry matter digestibility (IVDMD) and relative feed value (RFV) of 9 alfalfa accessions across 7 harvests following planting in October 2016 in Ethiopia.

Accession	Forage quality trait (% DM)								
	Ash	CP	NDF	ADF	ADL	Hemicel- lulose	Cellulose	IVDMD	RFV
ILRI_14176A	12.6	31.7bcd	38.6	21.7	5.7	16.8	16.0	82.2bc	179ab
ILRI_15585A	12.0	32.6bcd	38.7	20.6	5.9	18.1	14.8	83.8ab	177b
ILRI_5680A	13.2	27.4e	37.7	24.2	5.8	13.6	18.3	82.9bc	173b
ILRI_5681A	13.5	30.2d	40.8	24.6	6.1	16.1	18.6	81.3bc	160c
ILRI_7323A	12.9	33.2abc	37.5	22.4	5.3	15.1	17.1	82.1bc	178ab
ILRI_7369A	12.5	31.2cd	37.2	23.4	6.1	13.8	17.3	80.5c	177ab
ILRI_9234A	12.2	35.1a	38.5	21.2	5.5	17.3	15.7	87.0a	177b
ILRI_9237A	12.7	31.8bcd	36.6	21.7	5.1	14.8	16.6	83.0bc	184a
ILRI_9239A	12.4	33.8ab	37.3	21.8	5.6	15.4	16.3	83.3bc	180ab
LSD	NS	2.51	NS	NS	NS	NS	NS	3.31	6.98
CV (%)	5.1	4.5	10.0	8.6	13.6	15.0	9.1	2.3	2.3

Means within columns with a common letter are not significantly different ( $P>0.05$ ).

## Discussion

This study has shown that all alfalfa accessions grew satisfactorily during the period, producing acceptable yields of very high quality forage. Rainfall seemed to have a greater influence on growth rate of the various accessions than genetic differences, and timing of consecutive harvests appeared to be primarily dependent on weather conditions during the growth period. When sufficient moisture was present, i.e. during and immediately after the onset of the rainy season, plants grew more rapidly and flowered more quickly, as can be seen by the 30–45 day inter-harvest intervals for the 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> harvests in July, September, October and November 2017 (Figure 1). However, when weather conditions were less favorable for alfalfa growth, it took more than 90 days to reach the harvesting stage, i.e. 50% flowering. The long periods between harvests, from October to January and January to June in the establishment year and November to June in the second year, were associated with dry conditions in February and March. From these results we can infer that optimum yields throughout the year would be achievable only by applying irrigation water during periods of low rainfall.

The variations in growth and yield performance of different alfalfa accessions would be due to genetic differences between them and their response to environmental conditions experienced during the growing season, especially soil moisture. This finding concurs with those of previous reports, which show that environmental conditions play a significant role in the variation in dry matter yield among alfalfa cultivars (Veronesi et al. 2010). It has also been reported that cutting frequency has a significant

effect on forage yield and yield components in alfalfa (Tabacco et al. 2002; Borreani et al. 2006; Testa et al. 2011; Atis et al. 2019) and that the crop harvesting cycle has a significant effect on other parameters including stand height (Testa et al. 2011). Our harvest intervals were determined by flowering patterns, with 50% flowering being the criterion for harvesting. Since time of year has a significant impact on incidence of flowering, this factor also affected intervals between harvests, adding to the marked variation in this parameter throughout the year.

While the range of overall means for plant height of the various accessions across all harvests was 57.4 cm (ILRI\_14176A) to 70.0 cm (ILRI\_7323A), there was also significant ( $P<0.05$ ) variation in mean plant height of all accessions across harvests with stands at the 2<sup>nd</sup> (73.2 cm) and 5<sup>th</sup> (74.2 cm) harvests significantly taller than at all other harvests. The fact that the accessions took 140 days to reach this height before the second harvest and only 33 days to do so before the fifth harvest highlights the influence that time of year and soil moisture have on plant growth. Plant height is strongly associated with the total biomass yield of the crop (Tilly et al. 2013) and previous research has shown that plant height of alfalfa varies between cultivars (Djaman et al. 2020) as well as with crop management (Ullah et al. 2009) and soil fertility (Massaliyev et al. 2015). In our study, time of year and environmental conditions had a greater influence on plant height than the particular accession. Mean DM yields varied significantly ( $P<0.05$ ) among accessions and ranged from 3.86 t DM/ha for ILRI\_14176A to 4.99 t DM/ha for ILRI\_7323A. This was to be expected as previous studies have reported significant differences in DM yields among alfalfa cultivars, with yields of 2.0–3.3 t DM/ha (Yüksel et

al. 2016), and 2.6–3.6 t DM/ha (mean 3.2; [Altinok and Karakaya 2002](#)) under rain-fed conditions.

Seed yield improvement is critical for the commercial development of alfalfa varieties ([Huyghe et al. 2001](#)). Variation in seed production of alfalfa is a product of the genetic potential of the crop and environmental factors such as insect pollinator attractants ([Sreedhara et al. 2012](#)). In this experiment ILRI\_7323A, which performed best across the full range of agro-morphological traits, proved to have the highest relative seed yield, whereas ILRI\_9237A was the lowest seed-yielding accession (Table 4). Seed yields presented here, while similar to those reported by some researchers ([Al-Kahtani et al. 2017](#)), were significantly lower than those reported by others, although they were consistent in that genotypic variation affected seed yield over cutting periods ([Falcinelli 1999](#); [Bolaños-Aguilar et al. 2000](#); [Huyghe et al. 2001](#)).

Although some of the forage quality parameters, such as ash, NDF, ADF and cellulose concentrations, were not significantly different among the alfalfa accessions in the present experiment, significant variation among accessions was detected in this study for CP concentration, IVDMD and RFV. These results concur with the findings for alfalfa quality assessment by other researchers ([Milić et al. 2011](#)). Forages with a RFV of greater than 100 are generally considered to be of high quality ([Saha et al. 2010](#); [Schroeder 2013](#)) and all cultivars in our study had values above 150. In addition, according to the alfalfa quality rating of Orloff and Marble ([1997](#)), premium quality alfalfa forage contains 29% or less ADF. The results from our experiment demonstrate that, according to these criteria, all 9 accessions tested in our study would be considered to produce very high quality forage. In fact, since all accessions had CP concentration in excess of 30% at 50% flowering and IVDMD in excess of 80%, the forage could be considered of exceptionally high quality. The CP values are considered quite high but broadly similar to those reported by other scholars, e.g. Yolcu et al. ([2008](#)), who reported values of 24–32% for 12 cultivars and Awad and Bakri ([2009](#)), who reported values of 20–27% for 3 cultivars.

## Conclusion and Recommendations

In this study, the 9 alfalfa accessions achieved very satisfactory yields (27–35 t DM/ha) of very high quality forage (>80% IVDMD, >30% CP) during the 20 months following planting in a 544 mm rainfall environment. The effects of harvest and accession were significant for plant height and DM yield, which indicated genotype differences under a single management system. The superior performance of accession ILRI\_7323A in terms

of growth, seed yield and overall forage quality demonstrated its potential value for production in the region. Further studies are warranted to determine longevity of the stands and the improvement in yields in higher rainfall environments or when irrigated.

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(Note of the editors: All hyperlinks were verified 4 December 2020.)

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